THE EFFECT OF EASTERN REDCEDAR AND PRESCRIBED BURNING ON THE SOIL ECOSYSTEM OF THE CROSS TIMBERS FOREST

By

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CHAPTER I

INTRODUCTION

In terrestrial ecosystems soil microbes are the key drivers of biogeochemical cycles. Decomposition of organic matter by soil bacteria and fungi provides a large source of available nutrients in forest ecosystems (McGuire and Treseder, 2010). As the major donors of organic matter to soil, plants alter nutrient cycling through changes in nutrient content of their litter. Soil microbial communities are often defined by the chemical makeup of litterfall from plants above (Meier and Bowman, 2008; Talbot and Finzi, 2008). Plants and soil microbes are inherently tied together by the transfer of carbon through organic matter and nutrients mineralized through decomposition. Disturbances, such as fire, can shape both plant and soil communities while affecting biogeochemical cycles as well (Wells *et al.*, 1979; Bowman *et al.*, 2009). Fire can be seen in the fossil record starting around 420 million years ago, allowing both plant and soil microbial communities to adapt to this disturbance (Scott and Glasspool, 2006). The availability of nutrients from the combustion of organic material is highly dependent on fire intensity, precipitation and soil texture (Wells *et al.*, 1979; Schlesinger, 1997). Repeated fires over time can shape an ecosystem to support hardy, fire-tolerant plant species that may rely on the disturbance for

regeneration. Fire-mediated shifts in plant communities can alter soil microbial communities through chemical and structural differences in plant material adapted for fire tolerance. Fire, plants, and soil microbes shape biogeochemical cycles by controlling the input, movement, and release of carbon and nutrients in a forest ecosystem.

The Cross Timbers is a mosaic of grassland, savanna, and forest that represents a transition zone between eastern deciduous forests and the Great Plains. It is a relatively xeric ecosystem dominated by *Quercus stellata* Wangenh. (post oak) and *Quercus marilandica* Münchh. (blackjack oak) (Duck and Fletcher, 1943; Rice and Penfound, 1959). Historically, the Cross Timbers of Oklahoma have experienced periodic drought and fire, allowing fire-tolerant oaks to establish and survive. With the removal of fire from this landscape, the Cross Timbers forest has become an increasingly dense, closed canopy forest (DeSantis *et al.*, 2010). Climate change predictions show the possibility of increased drought severity in Oklahoma, which could lead to greater water competition in these unusually dense Cross Timbers forests (Survey, 2009; Allen *et al.*, 2010). Increased water competition could potentially lead to the creation of gaps through tree mortality, increased fuel loads, and greater wildfire potential. To conserve the Cross Timbers forest, it is imperative to understand what species are causing the rapid increase in tree density and what potential benefits or consequences exist from managing areas with prescribed burning.

The encroachment of invasive plant species can have dramatic effects on a soil ecosystem through changes in litter chemistry and the soil microbial

community that they support. An invasive plant can alter nutrient cycling by directly affecting microbes involved in specific nutrient cycles, like nitrification (Hawkes *et al.*, 2005). With changes in nutrient pools, an invasive species may create a plant-soil feedback loop where it enhances the soil ecosystem for its own survival while inhibiting the growth of native species (Reinhart *et al.*, 2003; Callaway *et al.*, 2004). Historically, *Juniperus virginiana* L. (eastern redcedar) was found on infertile sites and rocky outcrops in the Cross Timbers forest where it was isolated from fire (Therrell and Stahle, 1998). Over the past 50 years eastern redcedar has increased dramatically in the Cross Timbers forest, and the proliferation of this tree is blamed on a reduction of fire (DeSantis *et al.*, 2010). Eastern redcedar has been primarily recognized as an invasive species encroaching onto the Great Plains (Owensby *et al.*, 1973; Bragg and Hulbert, 1976; Coppedge *et al.*, 2001). In grasslands, eastern redcedar alters plant community composition and species richness (Gehring and Bragg, 1992; Briggs *et al.*, 2002; Linneman and Palmer, 2006). Eastern redcedar affects nutrient cycling in grassland ecosystems through changes in litter nutrients and alterations to soil nutrient pools (Norris *et al.*, 2001; Norris *et al.*, 2007; McKinley and Blair, 2008; McKinley *et al.*, 2008). Previous research suggests that eastern redcedar has an effect on plant species composition and supports a different soil pH in comparison to post oak-blackjack oak forests (Coile, 1933; van Els, 2009). *Juniperus* species and other members of the Cupressaceae, including eastern redcedar, are known to support arbuscular mycorrhizal symbioses (Newman and Reddell, 1987). *Quercus* species including post oak are commonly known to

have symbiotic relationships with ectomycorrhizal (ECM) fungi and can be highly dependent on them for survival and growth (Marks and Kozlowski, 1973; Cavender-Bares *et al.*, 2009). Studies have shown that competitive effects between ECM and AM fungi may negatively affect their plant hosts and can affect members of the soil microbial community that play roles in nutrient cycling (Sylvia and Jarstfer, 1997; McHugh and Gehring, 2006; Phillips and Fahey, 2006). The increase in eastern redcedar could create greater competition for resources in a soil that is already xeric and relatively infertile. Eastern redcedar encroachment could lead to alterations in the soil ecosystem of the Cross Timbers forest through changes in plant communities, litter inputs, soil nutrients, and soil microbial communities that would affect oak survival and alter nutrient cycling throughout the ecosystem.

Prescribed burning in oak forests is used to conserve forest soils, watershed quality, wildlife habitat, and prevent the spread of pathogens (Goodrum *et al.*, 1971; Laatsch and Anderson, 2000; Van Lear *et al.*, 2000; Meentemeyer *et al.*, 2008). Like any type of fire, prescribed burns can have a variety of effects on the soil depending on soil texture, soil moisture, and vegetation available for fuel (Gonzalez-Perez *et al.*, 2004). Fire can also directly alter the soil microbial community involved in nutrient cycling. Initially, fire decreases microbial biomass through heat-induced death and toxic compounds produced through combustion (Certini, 2005). Less is known about the long-term impact of repeated fire on soil microbial communities. Fire exclusion has threatened the species composition and health of oak forests through a process

called “mesophication” where fire removal allows fire intolerant, non-oak species to establish (Nowacki and Abrams, 2008). The resulting plant community creates forest floor conditions that are not conducive to fire, allowing more fire-intolerant species to establish. “Mesophication” has been documented across the U.S., showing the need to utilize prescribed burning for restoration and conservation of oak forests (Alexander and Arthur, 2010; DeSantis *et al.*, 2010). However, variability in soil responses to burning validates the necessity to study the impact of managing with fire at local scales and at varying burn frequencies.

The overall goal of the research reported here was to provide information about the potential effects of two major ecosystem forces in upland oak forests, encroachment of eastern redcedar and alteration of the fire regime, on ecosystem function. More specifically, I investigated how these two changes may affect biogeochemical cycles through their effects on soil microbial populations. My approach was to measure litter, soil nutrients, and soil microbial communities across various stages of eastern redcedar encroachment and various fire frequencies. The first study involved sampling oak forests encroached by eastern redcedar. Eight post oak-blackjack oak forests were sampled with different levels of eastern redcedar encroachment: 1) post oak-dominated 2) a mixture of post oak-eastern redcedar and 3) eastern redcedar-dominated. The second study compared forest stands that have been prescribed burned for the past 20 years at the rates of 0, 2.5, and 5 fires per decade. The perceived benefits of this research are a more profound understanding of changes in ecosystem function caused by eastern redcedar encroachment and

fire. This is essential information for assessment of changes caused by encroachment of invasive species and suppression of fire on biogeochemical cycles that could have long-term consequences for ecosystems.

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CHAPTER II

THE INFLUENCE OF*JUNIPERUS VIRGINIANA* ON LITTER AND THE SOIL ENVIRONMENT IN A *QUERCUS*- DOMINATED FOREST

ABSTRACT

Juniperus virginiana has increased greatly in basal area and tree density within the Cross Timbers of Oklahoma, a traditionally oak-dominated upland forest. This species is known to affect carbon and nitrogen cycling when invading grasslands and could cause shifts in soil microbial communities through its symbiotic association with arbuscular mycorrhizal (AM) fungi. My objective was to determine the impact of *J. virginiana* on the soil ecosystem of the Cross Timbers. I analyzed understory plant communities, litter nutrients, soil physiochemical properties, and soil microbial communities through phospholipid fatty acid analysis (PLFA). Plots dominated by eastern redcedar had a lower litter carbon concentration than post oak-dominated plots, while mixed species plots had a lower litter nitrogen concentration than oak-dominated plots. I did not observe any effect of tree species on soil physiochemical properties. Partial principle component analysis and partial redundancy analysis indicated differentiation of soil microbial community composition under each tree species. AM fungal biomarkers were notably higher under eastern redcedar than post oak. Alterations to the soil ecosystem may lead to concomitant shifts in nutrient

cycling within the forest, while the increase in AM fungi may give eastern redcedar a competitive advantage over historically dominant post oak.

INTRODUCTION

Interspecific differences exist beneath forest tree canopies that support specific soil physiochemical properties (Augusto *et al.*, 2002; Turk *et al.*, 2008; Kamei *et al.*, 2009) and soil microbial communities (Grayston and Prescott, 2005; Bach *et al.*, 2008; Mitchell *et al.*, 2010). In forests that are a mix of hardwoods and coniferous trees, the influence of individual species on the soil ecosystem has been studied (Saetre, 1999; Li and Han, 2008; Turk *et al.*, 2008; Ushio *et al.*, 2008). Differences between soil microbial communities and soil physiochemical properties can be indirectly influenced by the aboveground species through litter inputs (Sariyildiz and Anderson, 2003) as these effects may be strongly associated with the chemical makeup of the litter (Meier and Bowman, 2008; Talbot and Finzi, 2008).

The Cross Timbers forest is a relatively xeric ecosystem dominated by *Quercus stellata* Wangenh. (post oak) and to a lesser extent, *Quercus marilandica* Münchh. (blackjack oak) (Duck and Fletcher, 1943; Rice and Penfound, 1959). *Juniperus virginiana* L. (eastern redcedar) is a coniferous tree species that is encroaching throughout the Great Plains (Owensby *et al.*, 1973; Bragg and Hulbert, 1976; Coppedge *et al.*, 2001). Historically, eastern redcedar was found on infertile sites and rocky outcrops in the Cross Timbers forest where it was isolated from fire (Therrell and Stahle, 1998). In many Oklahoma Cross

Timbers stands eastern redcedar has established, increased greatly in basal area, and is becoming a dominant tree species in several regions (DeSantis *et al.*, 2010). The extent and impact of this conifer on the hardwood-dominated Cross Timbers forest has been largely unstudied.

Eastern redcedar invasion into grasslands alters plant community composition by decreasing grass species, increasing woody species, and decreasing plant species richness (Gehring and Bragg, 1992; Briggs *et al.*, 2002; Linneman and Palmer, 2006). Previous research suggests that in the Cross Timbers forest, species richness decreases close to eastern redcedar trunks, and plant communities are dominated by woody vines and tree seedlings (van Els, 2009). The development of stands dominated by eastern redcedar in the Cross Timbers could affect species composition and richness dramatically. Eastern redcedar affects nutrient cycling in grassland ecosystems through litter nutrient concentrations (Norris *et al.*, 2001, 2007) and soil nutrient pools (Norris *et al.*, 2007; McKinley and Blair, 2008; McKinley *et al.*, 2008). In a previous study comparing forest types, soil pH was 6.0 to 6.1 in post oak-blackjack oak forests and 6.2-6.7 in eastern redcedar forests in the surface 20 cm (Coile, 1933). Changes in understory communities, litter nutrients, and soil chemical properties mediated by eastern redcedar may dramatically change nutrient cycling in the Cross Timbers forest.

Juniperus species, such as eastern redcedar and other members of the Cupressaceae, are known to have a symbiotic relationship with arbuscular mycorrhizae (AM) fungi (Newman and Reddell, 1987). These fungal symbionts

are key players in ecosystem function and can shape both plant and soil microbial communities (Rillig and Klironomos, 2004; Cheng and Baumgartner, 2006; Rillig *et al.*, 2006). Oak species including post oak typically form associations with ectomycorrhizal (ECM) fungi and may be highly dependent on them for survival and growth (Marks and Kozlowski, 1973; Cavender-Bares *et al.*, 2009). Like AM fungi, ECM fungi assist with nutrient uptake and can play an important role in nutrient cycling (Smith and Read, 1997;Read and Perez-Moreno, 2003; Karliński *et al.*, 2007). Studies have shown that competitive effects between ECM and AM fungi may negatively affect host plant fitness and survival (Sylvia and Jarstfer, 1997; McHugh and Gehring, 2006). It has also been found that AM and ECM fungi can have differential effects on soil microbial communities, thereby altering members of the community involved in nutrient cycling (Phillips and Fahey, 2006). Therefore, an increase in abundance of the AM fungal cohort, eastern redcedar, may negatively affect post oak through competitive interactions with ECM fungi, the corresponding soil microbial communities, and nutrient availability. These belowground threats to post oak could cause dramatic changes in the historically oak-dominated Cross Timbers.

Based on previous studies, eastern redcedar could have a substantial effect on the overall ecology and ecosystem function of Cross Timbers forests. Changes in litter may cause concomitant alterations in soil chemical characteristics and soil microbial nutrient cycling. These alterations may be more conducive to eastern redcedar propagation and detrimental to the historical dominant tree of the Cross Timbers. The objective of this study was to assess

potential effects of eastern redcedar encroachment on the Cross Timbers forest through four different aspects: understory plant communities, litter quality, soil nutrients, and soil microbial communities. We hypothesized that these parameters would be altered by eastern redcedar encroachment into the Cross Timbers. We characterized soil physiochemical properties, litter nutrients, and soil microbial communities under redcedar dominated canopies, post oak dominated canopies, and in areas with both species. We hypothesized that soil nutrients would be highly correlated with differences in litter nutrient concentrations. We also hypothesized that soil microbial communities, especially AM fungal abundance, would correlate with the overstory tree species.

STUDY AREA

Forests used in this study were selected from 30 sampled in the 1950's by Rice and Penfound (1959) and resurveyed in 2007 (Desantis *et al.*, 2010). We selected eight stands from the 2007 study based on the overstory species composition. The stands were originally dominated by post oak and had substantial encroachment by eastern redcedar. The location of forest stands used in our current study ranged from central to western Oklahoma near the panhandle (Figure 1). These stands had a range of soil types from fine sands to sandy fine loams (Table 1).

METHODS

Plot establishment

At each site, we placed 2 plots for each of the following 3 classifications: 1. *J. virginiana* > 70%, 2. *Q. stellata* > 70%, and 3. *J. virginiana* and *Q. stellata* < 60% and > 40%. Plots were selected based on the species composition of a prism plot using a basal area factor (BAF) 10 prism.

Understory vegetation and litter measurement

At each plot, we placed a 1 m^2 quadrat. Herbaceous vegetation was identified to either genus or species level and percent cover was estimated using a customized Braun-Blanquet cover scale (Kent and Coker, 1992). Species that could not be identified in field were collected and identified in the laboratory. All litter was removed down to mineral soil in the four corners and center of the quadrat in an area approximately 8 cm in diameter. Litter was stored in bags for transport, washed gently for 30 seconds to remove mineral soil, and dried at 70° C for 48 hours. Dried litter samples were finely ground and analyzed by the Soil, Water, and Forage Analytical Laboratory (SWFAL) at Oklahoma State University for total carbon (TC), total nitrogen (TN), and lignin content. Carbon and nitrogen were measured through dry combustion (Bremner, 1996; Nelson and Sommers, 1996). Lignin was quantified after the determination of acid detergent fiber and digestion with 72% sulfuric acid (ANKOM Technology, 2002).

Soil collection and measurement

After removing all the litter to mineral soil, we took cores to a depth of 10 cm with an 8 cm diameter core sampler. The 5 cores taken at each plot were homogenized by hand, and a subsample was taken for chemical and soil microbial analyses. Two more soil cores were taken within the quadrat to measure bulk density and soil moisture. The soil was sieved with a 4mm and 2mm screen to remove root fragments and rocks. For chemical analysis, samples were analyzed by the SWFAL at Oklahoma State University for pH, total nitrogen (TN), soil organic matter (SOM), total soil organic carbon (SOC), NO_3-N, NH_4-N, total soil phosphorous (P), and total soil potassium (K) (Gavlak *et al.*, 2003). The SWFAL analyzed each sample twice, and we averaged the reported values at the subsample level. For bulk density and soil moisture, soil cores were weighed before and after drying at 105℃ for 4 8 hours. Carbon and nitrogen were measured through dry combustion (Bremner, 1996; Nelson and Sommers, 1996).

PLFA

Soil was freeze-dried and finely ground with a mortar and pestle. Five grams of each sample were mixed with a phosphate buffer, methanol, and chloroform for lipid extraction. The soil-solvent mixture was separated by centrifugation and then decanted with 1:2 mix of chloroform and methanol. Phosphate buffer was added and left for phase separation to occur overnight. After phase separation, the chloroform layer containing the lipids was recovered

and reduced by nitrogen flow at 50˚C. The lipids were separated into neutral lipids, glycolipids, and phospholipids by solid phase extraction by eluting with chloroform, acetone, and methanol, respectively. Phospholipids were hydrolized and methylated. The methylated fatty acids were extracted with hexane and evaporated under nitrogen at 37˚C. Phospholipid fatty acid (PLFA) analysis was performed using an Agilent 7890A gas chromatograph with an Agilent 5975C series mass selective detector. The peaks analyzed were for the following compounds: C16:1 ω 5 for AM fungi (Olsson *et al.*, 1995); 3-OH C14:0, C16:1 ω 9, C17:0 Δ 9,10, 2-OH C16:0, C18:1 ω 9 trans, cyC19:0 ω Δ 9,10 for gram negative bacteria (Wilkinson, 1988; Zelles, 1997); i-C15:0, a-C15:0, i-C16:0, i-C17:0 for gram positive bacteria (Harwood and Russell, 1984); C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 for non-specific bacteria (White *et al.*, 1996); C18:2 ω 9,12, C18:1 ω 9cis for saprophytic fungi (Karliński *et al.*, 2007).

Data Analysis

Phosphorus, K, NH_4-N, and NO_3-N were log transformed prior to analysis (Palmer, 1993). We used a split-plot model that averaged replicate plots within a site using a mixed-effects model to test the differences between understory plant functional groups, litter nutrients, soil physiochemical properties, and soil microbial communities across the plot types (SAS Institute, 2008). The same model was used to determine the differences between the western and central regions of the state, independent of dominant tree cover. Results of statistical tests were considered significant at α=0.05. Partial principle component analysis (pPCA) and partial redundancy analysis (pRDA) was conducted using CANOCO

where replicate plots were averaged at each site and covariables represented each site (ter Braak and Šmilauer, 2002). For the results and discussion, plot types are referred to as Redcedar for eastern redcedar dominated-plots, Mixed for eastern redcedar and post oak mixed plots, and Oak for post oak dominated-plots.

RESULTS

UNDERSTORY

We observed no differences in understory community composition under different canopy types. Woody plants composed 38%, grasses composed 5%, forbs composed 3%, and legumes composed 2% of the total cover when averaged across all plots (Table 2). Forbs, graminoid, and legume species cover was usually 5 to 10 times lower than woody plant cover within the plots.

LITTER NUTRIENTS

Oak litter had a higher TN percentage than Mixed litter, while Redcedar litter TN was not different from either Oak and Mixed litter TN (Table 3). The percent of TC in the litter was greater in Oak litter than in either Redcedar and Mixed litter. Dominant tree species cover appeared to have no effect on litter C:N ratio (30.7, P=0.5201), lignin content (29.0%, P=0.6141) or lignin:N ratio (26.4, P=0.4057). We failed to observe differences in litter nutrients the western and central regions (data not shown).

SOIL PHYSIOCHEMICAL PROPERTIES

We did not observe any effect of tree cover or region on any of the soil physiochemical properties (Table 4). In general, soil pH was slightly less than neutral. Bulk density averaged close to 1.0 g cm^{-3}, and the percent moisture averaged approximately 9%. Soil organic matter averaged 4% and TN was 0.17%. Phosphorus averaged close to 4.19 g m^{-2}, and K averaged 25.54 g m^{-2} across all plots. Nitrate averaged slightly lower than NH_4-N (1.06 and 2.19 g m^{-2} respectively).

PLFA

The mean PLFA values are represented in Table 5. Of the 19 PLFA markers, 4 were significantly different among Oak, Mixed, and Redcedar. The gram negative bacteria marker, C17:0 Δ 9,10, was 16% lower under Redcedar than Oak (*P*=0.0002). Two nonspecific bacteria markers, C14:0 and C20:0, were 66% and 96% higher under Redcedar than Oak (*P*=0.0033 and *P*=0.031 respectively). The marker for AM fungi, C16:1ω5, was 54% higher under Redcedar than Oak (*P*=0.0172), while the total for saprophytic fungi was not observed to be significantly different (*P*=0.3349). Totals for gram negative, gram positive and nonspecific bacterial markers showed no effect of canopy type. The total for saprophytic fungi showed no differences between canopy types. There were also no differences found between total PLFA mass, bacterial PLFA mass, and fungal PLFA between canopy types.

Two PLFA markers were significantly different between the central and western stands surveyed in this study. One gram negative bacterial marker, 2-OH C16:0, was 35% higher in west (P=0.03), while another, C18:1 ω 9cis, was 11% lower in the west than the central region of the state (P=0.043). The total of saprophytic fungal markers was higher in the central region than in the west (P=0.0204). Saprophytic fungal PLFAs were 15% less abundant in the west than in central forest stands. There were no significant differences observed for total PLFA mass, bacterial PLFA mass, or fungal PLFA mass between central and western plots.

The partial principal component analysis (pPCA) suggested that there was differentiation in soil microbial community composition under Oak, Mixed, and Redcedar (Figure 2). When designating the samples based on plot type, there was a shift from left to right along the first axis representing the different stages of eastern redcedar encroachment. The pPCA indicated that Oak and Redcedar had exclusive microbial community compositions, while Mixed soil microbial communities were a transition between Oak and Redcedar. Partial redundancy analysis (pRDA) displayed the increase in certain PLFA biomarkers based on each plot type (Figure 3). Notably C16:1ω5, C14:0, and C20:0 increased towards Redcedar while C17:0Δ9,10 increased towards Oak. These increases in PLFA biomarkers were also supported by their significantly different means as seen in Table 5.

DISCUSSION

We found that eastern redcedar fosters a different soil environment and produces litter that is chemically different from the dominant oak in the Cross Timbers forest. Different tree species within the same ecosystem are known to foster different soil microbial communities beneath their canopy (Grayston and Prescott, 2005; Bach *et al.*, 2008; Mitchell *et al.*, 2010). Differences in overstory are often reflected in soil physiochemical properties (Augusto *et al.*, 2002; Turk *et al.*, 2008; Kamei *et al.*, 2009) and litter chemistry (Sariyildiz and Anderson, 2003; Meier and Bowman, 2008; Talbot and Finzi, 2008). Although we did not find a connection between overstory and soil nutrients, differences in litter and soil microbial communities have the potential to alter carbon and nitrogen cycling and influence species composition in oak-dominated Cross Timbers forests.

Eastern redcedar affects nutrient cycling in grassland ecosystems through litter nutrient concentrations and soil nutrient pools (Norris *et al.*, 2001, 2007; McKinley and Blair, 2008; McKinley *et al.*, 2008). In comparison to Oak, Redcedar had a lower litter carbon concentration. However, this difference in litter carbon content was not reflected in soil organic carbon. The discrepancy between aboveground litter carbon and soil carbon could be explained by three different scenarios. First, root exudates and root decomposition may compose a large part of the soil organic carbon pool, thereby minimizing differences in litter carbon input. Second, litter fall and litter decomposition rates may be slower under eastern redcedar than under post oak. With more carbon-rich litter deposition under oaks, there should be a greater amount of organic carbon

available to enter the soil pool. However if during decomposition carbon is respired from oak litter than eastern redcedar litter at a faster rate, an equivalent amount of carbon may enter the soil pool. The litter C:N ratio was not significantly different between Oak and Redcedar, indicating similar rates of decomposition. The lignin:N ratio, an indicator of decomposition rates through N immobilization, was also not significantly different. Other nutrients or secondary compounds not measured in this study may limit eastern redcedar litter decomposition, allowing for slower transfer of carbon between litter and soil pools. Third, greater amounts of AM fungi in soil under redcedar may make up the difference in litter carbon contributions to the soil pool. Fungal PLFA mass was not significantly different between Oak and Redcedar, but compounds produced by AM fungi could play some role. AM fungi produces a glycoprotein called glomalin that is associated with greater amounts of fungal hyphae, soil aggregation, and increases in soil organic carbon (Rillig *et al.*, 2001; Wilson *et al*. 2009). It is not clear what made up the difference between aboveground and belowground carbon, and this discrepancy requires further investigation. It is also important to note the differences in TN among Mixed, Oak, and Redcedar. Oak had significantly higher TN in the litter than Mixed, but neither were significantly different from Redcedar. Nitrogen constituents may be immobilized from the litter at a higher rate under Mixed canopies than under Oak. Differences in litter carbon and nitrogen could potentially affect mineralization of nitrogen and decomposition of organic matter in the soil of the Cross Timbers.

Analysis of soil microbial communities between central and western regions demonstrated significant variability. The major difference in the soil communities were the increase in abundance of saprophytic fungal PLFAs; saprophytic fungi were 14% higher in the central region overall. Saprophytic fungi may be dependent on an environmental gradient like precipitation or differences in litter quality not measured in this study. Differences in this fungal community may lead to faster turnover of soil organic matter independent of canopy tree species in the central region of the Cross Timbers. Slower decomposition of soil organic matter in the west may give these Cross Timbers stands the potential to be greater carbon sinks and increasingly N-limited.

The largest difference in soil microbial community structure between Oak and Redcedar was AM fungal abundance. This fungal symbiont assists its host in nutrient acquisition and may provide a competitive advantage against local, non-AM symbiotic plant species. The bacterial markers that vary in abundance between the cover types may be influenced directly by eastern redcedar or indirectly through the fungal symbiont. It is important to note that some bacterial PLFAs were lower when AM fungal abundances were higher. This could be a direct influence of the fungi or a competitive interaction between the two groups within the soil environment. The potential competitive advantage provided to eastern redcedar through the mycorrhizal symbiosis could be a future issue in this upland oak forest. Eastern redcedar has increased more than 5-fold in basal area within the Cross Timbers forests in the past 50 years (DeSantis *et al.*, 2010). A possible mechanism for the dramatic increase in eastern redcedar is its

association with AM fungi. The increase in AM fungi in the soil could increase the establishment of eastern redcedar while impeding the establishment of other, non-AM symbiotic plant species.

Our results show that eastern redcedar fosters a greatly different soil environment than is normally found in oak dominated forests. The change in species composition in the overstory of the Cross Timbers is unfavorable for conserving an ecosystem that may be one of the largest areas of old-growth forest in the eastern United States (Therrell and Stahle, 1998). Management in these areas should assess the local consequences of eastern redcedar encroachment and take into account the possible legacy the species will have on the soil. Our research indicates large-scale management decisions may provide mixed results due to regional differences in the Cross Timbers soil ecosystem. If the Cross Timbers as a whole become more similar to the mixed species plots, alterations to nitrogen and carbon cycling may occur due to lower litter nitrogen concentration. Several of these forest areas rarely burn, allowing the thick, slowly decomposing litter layer to act as a carbon sink. If eastern redcedar becomes more dominant, the forest floor may not store as much carbon in the litter as it did previously. The reintroduction of fire to control eastern redcedar seedlings and mechanical removal of large individuals seems to be the only way to maintain the oak-dominated canopy and its carbon-rich litter. If this management practice is used, carbon losses from the ignition of litter must be considered as well. Changes in soil microbial communities, especially AM fungi, may give a competitive edge to the rapidly increasing eastern redcedar.

Alterations to the soil at the microbial community level may be harmful to the long-term survival of oaks. Even if redcedar is removed, residual AM fungi and the associated soil microbial community may impede oak survival. The increase in AM fungi will not only accelerate the success of eastern redcedar encroachment, but may also allow the establishment of other invasive species with AM fungi associations. Belowground changes in the Cross Timbers should be a concern for both researchers and land managers when addressing conservation issues in this forest ecosystem. Without any removal or control of eastern redcedar, the Cross Timbers will become increasingly different from its oak-dominated history.

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Table 1.Soil types and textures for all *Quercus*-dominated forest sites visited in the Cross Timbers of Oklahoma.

Site	County	Soil Type	Soil Texture
1	Hughes	Hector-Endsaw Complex	Fine Sandy Loam
2	Logan	Stephenville- Darsil Complex	Fine Sandy Loam
3	Payne	Derby Loamy Fine Sand	Loamy Fine Sand
4	Payne	Stephenville- Darsil Complex and Stephenville Fine Sandy Loam	Fine Sandy Loam
5	Blaine	Nobscot Fine Sand and Devol Fine Sandy Loam	Fine Sand and Fine Sandy Loam
6	Woodward	Nobscot Eda Complex	Fine Sand
7	Comanche	Rock Outcrop-Brico Complex and Brico Soils and Rocky Outcrop	Cobbly Loam and Very Cobbly Loam
8	Comanche	Brico Soils and Rocky Outcrop	Very Cobbly Loam

Table 2. Percent cover by functional group, SE= standard error.

Functional Group	Oak	SE	Mixed	SE	Redcedar	SE	*P*-value
Forbs	2.05	1.19	2.5	1.59	5.62	4.64	0.6361
Woody	44.66	14	36.07	11.86	34.74	11.59	0.5370
Grasses	2.46	2.46	4.07	3.59	7.9	2.28	0.4024
Legumes	1.61	1.19	1.61	1.19	1.17	1.17	0.9537
Total cover	50.78	12.89	44.24	11.84	49.43	14.94	0.7825

Table 3. Litter nutrients and ratios based on percent total nitrogen in percent of total mass. SE= standard error. Mean separation is displayed by letters next to mean values.

Litter Variables	Oak		SE	Mixed		SE	Redcedar		SE	*P*-value
%TN	1.18	*a*	0.05	1.06	*b*	0.04	1.12	*ab*	0.03	0.0462
%TC	36.03	*a*	0.56	31.70	*b*	0.26	33.08	*b*	0.53	<0.00001
C:N	31.49		1.76	30.55		1.25	30.01		1.08	0.5201
%Lignin	28.98		1.36	28.27		1.00	29.69		0.86	0.6141
Lignin:N	25.14		1.37	27.08		0.94	27.03		0.94	0.4057

Table 4. Soil physiochemical properties. P, K, NH_4-N, and NO_3-N are g m^{-2}. Bulk density is measured in g cm^{-3}.

Soil Variables	Oak	SE	Mixed	SE	Redcedar	SE	*P*-value
pH	6.19	0.20	6.53	0.32	6.94	0.13	0.0662
%SOM	3.31	0.45	4.99	0.70	4.32	0.75	0.1179
%SOC	1.92	0.28	2.90	0.42	2.50	0.38	0.1179
%TN	0.14	0.03	0.18	0.03	0.18	0.03	0.2522
P	4.13	0.72	4.26	0.87	4.19	0.92	0.9467
K	23.96	4.13	25.78	4.87	26.89	3.98	0.3168
NH_4-N	2.09	0.47	2.17	0.36	2.30	0.60	0.4257
NO_3-N	1.01	0.31	0.98	0.25	1.19	0.27	0.4257
C:N	16.09	1.58	17.60	2.52	14.72	1.91	0.6334
%water	8.02	1.25	9.10	1.59	10.14	2.29	0.1886
Bulk Density	1.04	0.03	0.97	0.04	1.01	0.05	0.3754

Table 5. PLFA biomarker values for each marker and composite values. GNB represents gram negative bacteria, GPB represents gram positive bacteria, NSB represents non-specific bacteria, AM fungi represents arbuscular mycorrhizal fungi, and SAP fungi represents saprophytic fungi. Mean separation is displayed by letters next to mean values

Categories	Marker	Oak		SE	Mixed		SE	Redcedar		SE	*P*-value
GNB	**3-OH C14:0**	0.64		0.21	0.95		0.23	0.98		0.15	0.1010
GNB	**C16:1ω9**	5.85		0.36	6.54		0.55	5.99		0.37	0.2263
GNB	**C17:0 Δ 9,10**	3.19	*a*	0.12	3.02	*a*	0.11	2.67	*b*	0.13	0.0001
GNB	**2-OH C16:0**	2.24		0.37	2.28		0.27	2.30		0.23	0.9783
GNB	**C18:1ω9 trans**	1.42		0.10	1.60		0.11	1.45		0.22	0.6853
GNB	**cyC19:0ωΔ 9,10**	10.56		0.66	9.94		0.45	8.49		1.10	0.1219
GPB	**i-C15:0**	11.01		0.13	10.93		0.30	10.72		0.41	0.7590
GPB	**a-C15:0**	6.12		0.15	6.03		0.37	5.92		0.33	0.8526
GPB	**i-C16:0**	6.39		0.53	6.76		0.31	6.71		0.35	0.7627
GPB	**i-C17:0**	3.23		0.13	3.21		0.12	3.22		0.10	0.9944
NSB	**C14:0**	2.19	*a*	0.20	2.88	*a*	0.29	3.64	*b*	0.24	0.0033
NSB	**C15:0**	1.22		0.06	1.19		0.04	1.16		0.10	0.7953
NSB	**C16:0**	20.58		0.57	19.62		0.45	20.60		0.72	0.3344
NSB	**C17:0**	0.60		0.16	0.34		0.12	0.15		0.11	0.0929
NSB	**C18:0**	4.83		0.30	4.40		0.22	4.40		0.12	0.0621
NSB	**C20:0**	0.89	*a*	0.19	1.29	*ab*	0.13	1.74	*b*	0.26	0.0310
AM Fungi	**C16:1ω5**	3.74	*a*	0.27	4.19	*a*	0.17	5.65	*b*	0.66	0.0172

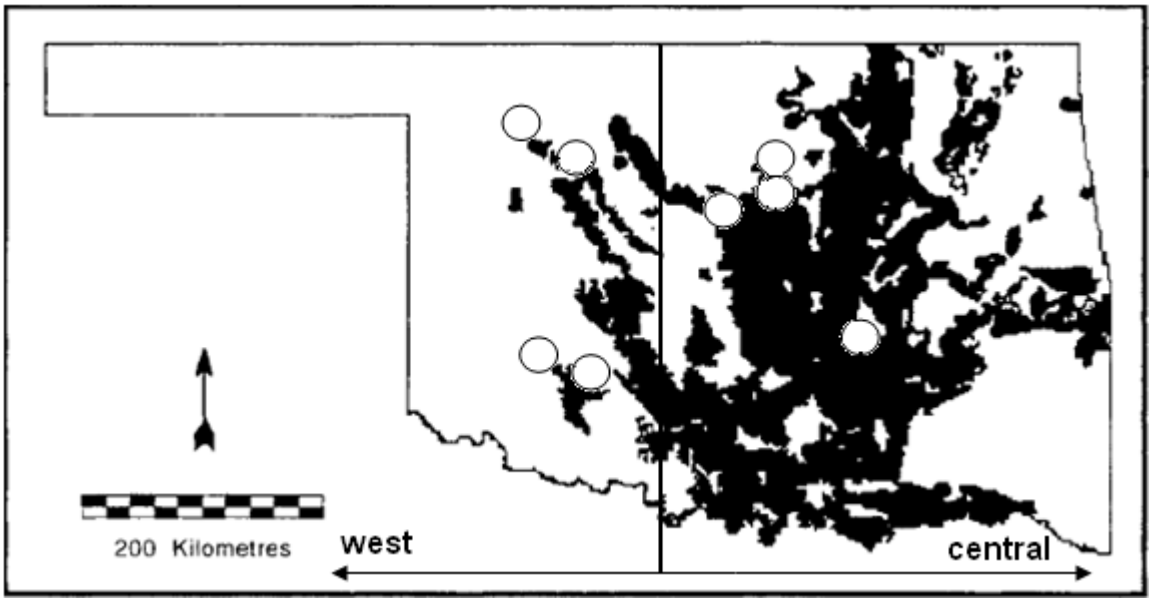
Table 5 continued.

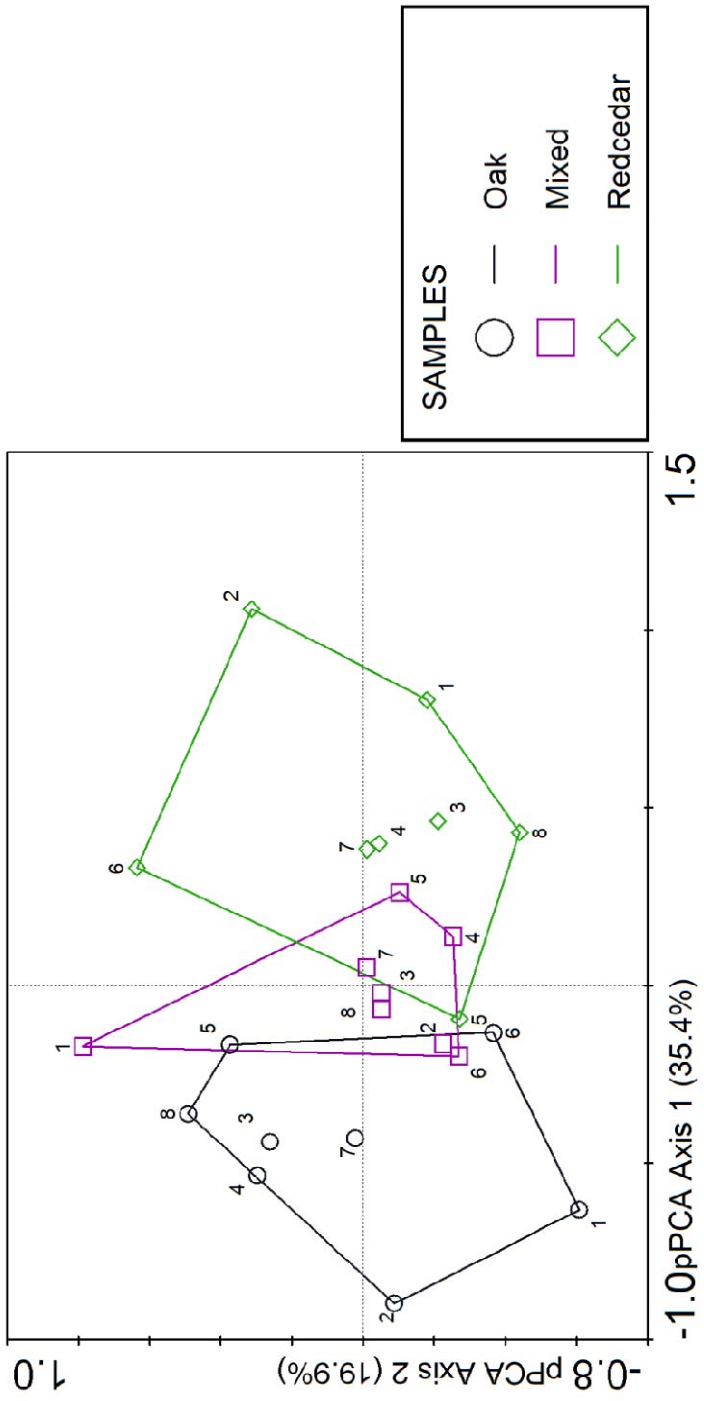
SAP Fungi	**C18:2 ω9,12**	3.42	0.37	3.67	0.48	3.62	0.41	0.7413
SAP Fungi	**C18:1ω9cis**	11.89	0.66	11.15	0.56	10.61	0.45	0.2101
Totals								
	GNB	23.90	0.80	24.34	0.69	21.86	1.41	0.1821
	GPB	26.74	0.63	26.94	0.86	26.57	0.93	0.9361
	NS	30.30	0.72	29.71	0.83	31.69	0.97	0.1458
	AM Fungi	3.74 *a*	0.27	4.19 *a*	0.17	5.65 *b*	0.66	0.0172
	SAP Fungi	15.31	0.76	14.82	0.73	14.23	0.52	0.3349
	bacterial	80.95	0.77	80.99	0.83	80.12	1.13	0.7131
	fungal	19.05	0.77	19.01	0.83	19.88	1.13	0.7131
	bacterial mass	3107.08	422.37	3570.23	388.37	3050.68	389.02	0.4623
	fungal mass	776.92	90.49	898.83	107.80	822.23	122.76	0.6660
	total mass	3884.00	510.52	4469.07	490.38	3872.91	504.00	0.5140

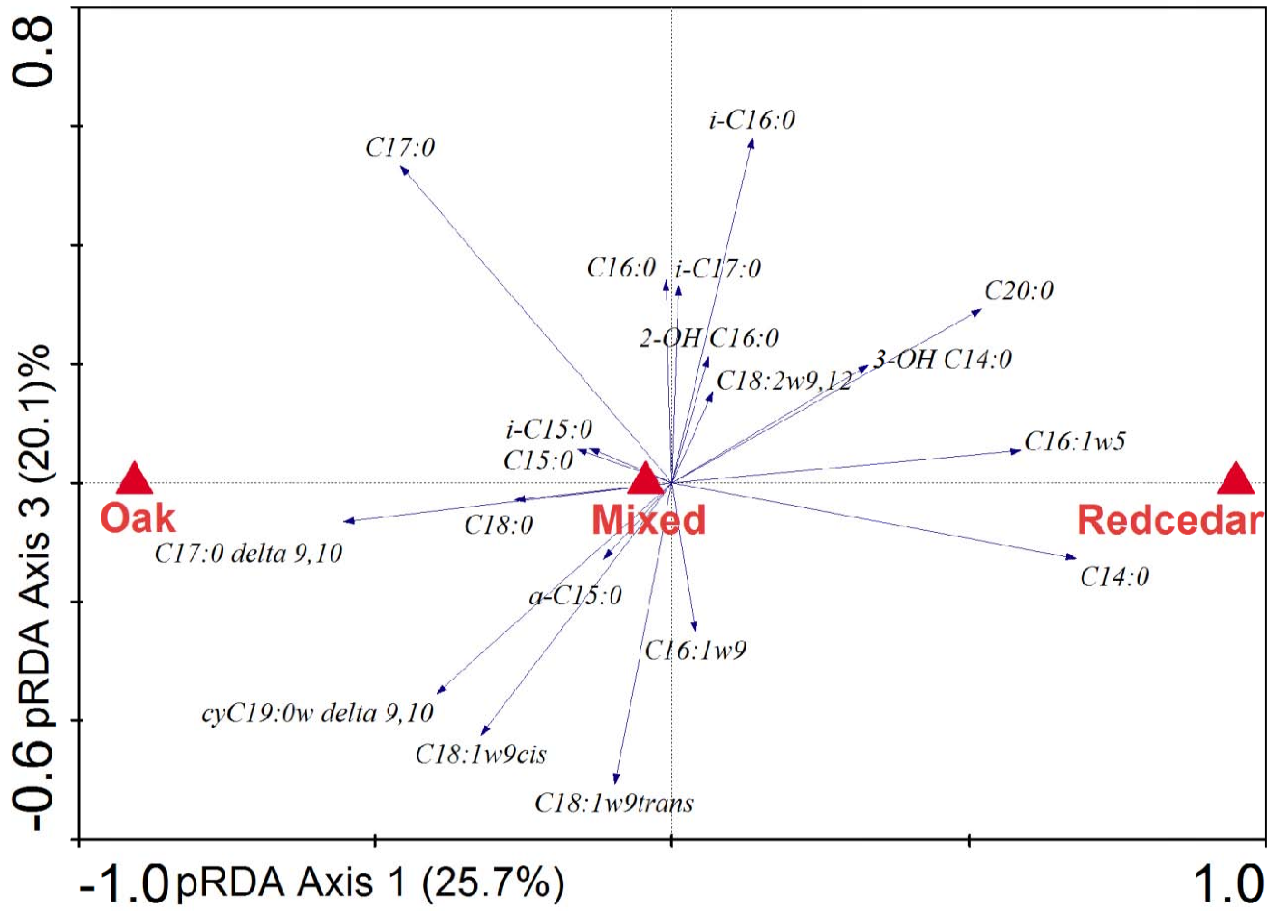
Figure 1. Map of Oklahoma with black area representing the Cross Timbers. White circles represent the locations of sites sampled for the study and are grouped into west and central regions. The map was taken from Duck and Fletcher (1943).

Figure 2. Partial principle component analysis (pPCA) displaying soil microbial community composition based on 19 PLFA biomarkers. The percentage of variation explained by axes is displayed by the respective axis. The numbers next to samples represent the sampling sites.

Figure 3. Partial redundancy analysis (pRDA) displaying the response of 19 PLFA biomarkers to three plot types. The percentage of variation explained by axes is displayed by the respective axis


200 Kilometres
west
central


SAMPLES
Oak
Mixed
Redcedar
1.0
-0.8 pPCA Axis 2 (19.9%)
-1.0
pPCA Axis 1 (35.4%)
1.5


0.8
-0.6
pRDA Axis 3 (20.1)%
-1.0
pRDA Axis 1 (25.7%)
1.0
i-C16:0
C17:0
C16:0
i-C17:0
C20:0
2-OH C16:0
3-OH C14:0
C18:2w9,12
i-C15:0
C15:0
C16:1w5
Oak
Mixed
Redcedar
C18:0
C17:0 delta 9,10
C14:0
a-C15:0
C16:1w9
cyC19:0w delta 9,10
C18:1w9cis
C18:1w9trans

CHAPTER III

THE EFFECT OF PRESCRIBED BURNING ON LITTER, SOIL, AND MICROBIAL COMMUNITIES IN AN UPLAND OAK FOREST

ABSTRACT

Prescribed burning is a forest management technique used to reduce fuel loads, prevent exotic species establishment, and maintain wildlife habitat. In recent years, managing forests for their carbon stocks has become increasingly important as a management goal. Prescribed burning can have various effects on the forest floor and soil that alter nutrient cycling throughout the ecosystem. We assessed how prescribed burning altered litter, soil physiochemical properties, and soil microbial communities within an upland oak forest. Soil samples were taken within management units in the Okmulgee Wildlife Management Area (OWMA) that are burned at 5, 2.5 and 0 fires per decade (FPD) over the past 20 years. Our results indicated burning at 5 FPD caused dramatic reductions in soil organic matter (SOM) and altered the soil microbial community, while burning at 2.5 and 5 FPD decreased litter N. For the soil characteristics pH, total N, NO_3-N, NH_4-N, P, K, and soil moisture, we failed to find differences among the treatments. We found that between 5 and 2.5 FPD, there was a 151% increase in SOM, 175% increase in soil organic carbon (SOC),

and a decrease in bulk density. Phospholipid fatty acid analysis (PLFA) indicated gram negative bacteria were 23% higher in 2.5 than 5 FPD. Both burn units had less total nitrogen (N) in the litter than the 0 FPD unit, resulting in higher C:N ratios. Similar soil characteristics between 0 and 2.5 FPD suggested that prescribed burning can be used as a management technique while maintaining the forest soil carbon pool. However, burning reduced litter N, creating a situation where the soil ecosystem may become N-limited.

INTRODUCTION

Prescribed burning maintains the health of oak forests by preventing spread of pathogens, removal of exotic species, and promoting oak regeneration (Laatsch and Anderson, 2000; Van Lear *et al.*, 2000; Meentemeyer *et al.*, 2008). From a wildlife perspective, maintaining an oak-dominated forest is especially important for acorn production and habitat maintenance (Goodrum *et al.*, 1971). Fire exclusion has threatened the stability of oak forests through a positive feedback loop referred to as “mesophication” (Nowacki and Abrams, 2008). This begins when fire suppression allows fire-intolerant, non-oak species to establish and create more closed canopy forests. While canopy closure increases, forest floor conditions become less conducive to the spread of fire. As fire suppression continues over time more shade-tolerant, fire intolerant species proliferate and exclude fire-tolerant, shade-intolerant oaks. “Mesophication” has been associated with the spread of red maple in eastern Kentucky oak forests (Alexander and Arthur, 2010), and an increase in woody, mesophytic species in the Cross Timbers forest of Oklahoma (DeSantis *et al.*, 2010). The

reintroduction of fire into oak forests is important to maintain both the health and the historical species composition of the ecosystem.

Ecosystem-based management in forests focuses on maintaining natural, diverse landscapes that can provide products or services to the public such as wildlife habitat, soil, conservation, watershed conservation, and mitigation of greenhouse gases (Scott *et al.*, 2006; Burger, 2009). Forests are an active part of the global carbon cycle by sequestering carbon through photosynthesis and adding carbon to soil organic matter (SOM) in recalcitrant forms. Carbon is released through the decomposition of organic matter and as the result of conventional management practices such as prescribed burning. Management of belowground carbon stocks is particularly important in old growth forests (Malhi *et al.*, 1999), and the Cross Timbers may be one of the largest areas of old-growth forest in the eastern United States (Therrell and Stahle, 1998). It is imperative to assess the effects of prescribed burning on the Cross Timbers as this ecosystem could potentially be managed to offset carbon emissions.

Burning affects the chemical and physical makeup of soil in a variety of ways. Fire effects on soil are highly dependent on its intensity; therefore, the differences between effects of wildfire and prescribed burning may be large. Soils respond differently to fires based on soil type, moisture, and type of aboveground plant materials (Gonzalez-Perez *et al.*, 2004). Soil organic matter can increase, decrease, or have no detectable change depending on the frequency of fire (Johnson, 1992; Phillips *et al.*, 2000; Neill *et al.*, 2007). When burning is halted, SOM and soil organic carbon (SOC) should eventually return to

unburned levels. The time it takes for the accrual of new SOM is highly dependent on environmental factors such as soil texture and precipitation, making general statements about forest soil recovery from fire anecdotal. Meta-analysis on ecosystem nitrogen response to fire suggested no significant effect on the concentration of N in forest floor fuels or on the total N concentration in soil (Wan *et al.*, 2001). However, like carbon, N transformations in the soil and litter are highly dependent on environmental variables and the intensity of the actual fire. Management may also prevent rapid accretion of SOM because of repetitive burning. Soil fertility responses to prescribed burning must be assessed at regional levels in order to properly manage forest ecosystems across a heterogeneous, broad landscape like the Cross Timbers forest.

Fire can have a variety of effects on the soil microbial community depending on site characteristics, fire intensity, and sampling method (Wells *et al.*, 1979). It is known that fire reduces soil microbial biomass and is especially detrimental to soil fungal biomass (Fritze *et al.*, 1993; Bååth *et al.*, 1995; Prieto-Fernandez *et al.*, 1998). Several studies have focused on the short-term effects of fire on soil microbial communities (Certini, 2005). The effect of fire on soil ecosystems has often been studied after only one burn (Pietikainen *et al.*, 2000; Esquilin *et al.*, 2007; Hamman *et al.*, 2007; Smith *et al.*, 2008). Less research has focused on forest ecosystems that have had repetitive prescribed burning for several years. Prescribed burning in forests has shown increases in gram negative and gram positive bacterial PLFA biomarkers, with a decrease in fungal PLFA biomarkers a year after two successive burns (Ponder *et al.*, 2009).

Managing forests with prescribed burning can play a large part in shaping soil microbial communities that are the major drivers of nutrient cycles in an ecosystem.

Though prescribed burning can affect soil fertility in a number of ways, it has an even greater effect on the forest floor. Predictive models and empirical data indicate that prescribed burning in forests has little effect on C:N ratios in the mineral soil and has greater effects on the organic layer above (Garten, 2006). Litter was greatly affected by prescribed burning through the reduction of the organic layer of the forest floor (Wells, 1971). Fire volatilized compounds in the organic layer, causing losses of both nitrogen and carbon constituents. After burning, the movement of C and N from the litter into the mineral soil is highly dependent on environmental factors such as precipitation and soil texture. The loss or addition of litter-based nutrients can have great effects on the nutrient cycling and fertility of a forest ecosystem. Previous studies have shown that N in the organic layer of the forest floor in repeatedly burned plots was lower than in unburned plots (Bell and Binkley, 1989; Hernandez and Hobbie, 2008). Prescribed burning can cause N-limitation in an ecosystem by volatilizing N through combustion of the litter. Less N becomes plant available in the soil pool, and future litter pools will become increasingly N-deficient (Reich *et al.*, 2001; Hernandez and Hobbie, 2008). It is possible that a repetitive management technique like prescribed burning could have detrimental effects on soil fertility and nutrient cycling due to long-term, repetitive litter combustion.

The objectives of this research were to determine the long-term effects of prescribed burning in xeric oak forests on the soil microbial community in order to understand how this management technique affects the driving force behind biogeochemical cycling. This study was performed in an upland oak forest that has undergone managed burning for over 20 years with low-intensity dormant season fires. The burns were conducted at the frequencies of 0, 2.5, and 5 fires per decade in large management units ranging from 100 to 600 ha in size. I measured the effects of fire on the forest floor, mineral soil, and soil microbial communities to test the following hypotheses: 1) litter, soil, and microbial characteristics are more similar in burned units than unburned units 2) the effect of burning increases with fire frequency and 3) shifts in litter and soil nutrients are reflected in changes in soil microbial communities.

STUDY AREA

The study was conducted at the Okmulgee Wildlife Management Area (OWMA), a 2,400 ha forest located in the eastern Cross Timbers under management of the Oklahoma Department of Wildlife Conservation. It is an upland, relatively xeric forest dominated by *Quercus stellata* and *Quercus marilandica* (Duck and Fletcher, 1943; Rice and Penfound, 1959). Soils were of the Hector-Endsaw complex which are well drained, shallow stony fine sandy loams (Sparwasser *et al.*, 1968). The study concerned three management units burned at the rate of 0, 2.5, and 5 fires per decade beginning in 1988. There were no records of fire prior to 1989. A study of fire scars in the area found a fire frequency of 5 fires per decade for the period of 1900 to 1989 (DeSantis, 2010).

Both burned units in the study were burned last in February 2007, three growing seasons prior to sampling.

METHODS

Plot determination

In June 2007 we randomly located five plots where overstory cover was >70% post oak in each of the three fire frequency treatments. The main interest was in learning the effects of prescribed burning in this post oak dominated forest.

Litter collection and measurement

At each plot a 1-m^2 quadrat was placed on the ground. All litter was removed from an 8 cm diameter circular area down to mineral soil in the four corners and center of the quadrat. The five litter subsamples were bulked into one sample. Litter was stored in bags for transport to the laboratory, washed gently for 30 seconds to remove mineral soil, and dried at 70°C for 48 hours. Dried litter samples were finely ground and analyzed by the Soil, Water, and Forage Analytical Laboratory (SWFAL) at Oklahoma State University for carbon, total nitrogen, and lignin content. Carbon and nitrogen were measured through dry combustion (Bremner, 1996; Nelson and Sommers, 1996). Lignin was quantified after the determination of acid detergent fiber and digestion with 72% sulfuric acid (ANKOM Technology, 2002).

Soil collection and measurement

After removing all the litter down to mineral soil, we took soil cores to a depth of 10 cm with an 8 cm diameter sampler. The 5 cores taken at each plot were homogenized, and a subsample for chemical analysis was taken. I took two more soil cores within the quadrat to measure bulk density and soil moisture. The soil was sieved with a 4mm and 2mm screen to remove root fragments and rocks. For chemical analysis, samples were analyzed by the SWFAL at Oklahoma State University for pH, total soil N, SOM, SOC, NO_3-N, NH_4-N, total soil P, and total soil K (Gavlak *et al.*, 2003). The SWFAL analyzed two sub-samples per field sample, and we averaged sub-sample values for statistical analysis. For bulk density and soil moisture, soil cores were weighed before and after drying at 105℃ for 48 hours. Carbon and nitr ogen were measured through dry combustion (Bremner, 1996; Nelson and Sommers, 1996).

PLFA

Soil was freeze dried and finely ground with a mortar and pestle. Five grams of each sample was mixed with a phosphate buffer, methanol, and chloroform for lipid extraction. The soil-solvent mixture was separated by centrifugation and then decanted with 1:2 mix of chloroform and methanol. Phosphate buffer was added and left for phase separation to occur overnight. After phase separation, the chloroform layer containing the lipids was recovered and reduced by nitrogen flow at 50˚C. The lipids were separated into neutral lipids, glycolipids, and phospholipids by solid phase extraction by eluting with

chloroform, acetone, and methanol, respectively. Phospholipids were hydrolized and methylated. The methylated fatty acids were extracted with hexane and evaporated under nitrogen at 37˚C. Phospholipid fatty acid (PLFA) analysis was performed using an Agilent 7890A gas chromatograph with an Agilent 5975C series mass selective detector. The peaks analyzed were for the following compounds: C16:1 ω 5 for AM fungi (Olsson *et al.*, 1995); 3-OH C14:0, C16:1 ω 9, C17:0 Δ 9,10, 2-OH C16:0, C18:1 ω 9 trans, cyC19:0 ω Δ 9,10 for gram negative bacteria (Wilkinson, 1988; Zelles, 1997); i-C15:0, a-C15:0, i-C16:0, i-C17:0 for gram positive bacteria (Harwood and Russell, 1984); C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 for non-specific bacteria (White *et al.*, 1996); C18:2 ω 9,12, C18:1 ω 9cis for saprophytic fungi (Karlinski *et al.*, 2007).

Data Analysis

Phosphorus, K, NH_4-N, and NO_3-N were log transformed prior to analysis (Palmer, 1993). We used a general linear model to test significance of fire frequency effects on litter nutrients, soil physiochemical properties, and soil microbial communities (SAS Institute, 2008). Results of statistical tests were considered significant at P=0.05.

RESULTS

Litter Nutrients

Total nitrogen was 26% higher in 0 FPD than 2.5 and 5 FPD treatments (P=0.0017, Figure 1). Total carbon and lignin content were not significantly different between the fire frequencies (P=0.8021 and P=0.3517, respectively).

The differences in total nitrogen led to significant differences in the C:N ratios among the fire frequencies (*P*=0.0039). The C:N ratio was 28% and 25% higher in 2.5 and 5 FPD than 0 FPD (Figure 1). The lignin:N ratio was significantly different between 2.5 and 0 FPD (Figure 1, *P*=0.019). The 2.5 FPD lignin:N ratio was 28% higher than the 0 FPD lignin:N ratio.

Soil Nutrients

We did not observe pH, total N, NO_3-N, NH_4-N, P, K, and soil moisture to be significantly affected by the fire frequencies. On average, soil pH was 4.8 and the soil moisture content was 11.8% by mass. Total nitrogen averaged 0.12%, NO_3-N was 0.22 g m^{-2}, and NH_4-N was 2.70 g m^{-2}. Phosphorus and K averaged 3.13 and 13.99 g m^{-2} respectively. Percent SOM and SOC were significantly lower in 5 FPD than 2.5 and 0 FPD (*P*=0.0383). The 0 FPD and 2.5 FPD frequencies had approximately 151% more SOM and 175% more SOC than the 5 FPD unit (Figure 2). Bulk density was also significantly greater in 5 FPD than 0 and 2.5 FPD treatments (*P*=0.0039, Figure 2). Fire did not appear to significantly affect the C:N ratio, which averaged 24.29 (*P*=0.1630)

Soil PLFA

Soil PLFAs were dominated by bacterial biomarkers that usually consisted of approximately 75% of all PLFAs. None of the PLFA markers showed an effect of fire frequency except cyC19:0 ω Δ 9,10, a gram negative bacteria marker (*P*=0.0182). The total of arbuscular mycorrhizal fungi and saprophytic fungal markers were not found to be significantly different among fire frequencies

(*P*=0.4047 and *P*=0.7378, respectively). Total non-specific and gram positive bacteria biomarkers were not found to be significantly different among the fire frequencies (*P*=0.1369 and *P*=0.1664, respectively). The total for gram negative bacteria biomarkers was 23% higher in 0 FPD and 22% higher in 2.5 FPD than the 5 FPD frequency (*P*=0.0378, Figure 3). Total PLFA, fungal PLFA, and bacterial PLFA mass were not observed as significantly different among the fire frequencies (*P*=0.2212, *P*=0.2936, and *P*=0.2141, respectively).

DISCUSSION

Our results indicated burning at a high frequency of 5 FPD maintained a different chemical and biological environment in the mineral soil than at 0 and 2.5 FPD. The organic layer of the forest floor responded to any burn frequency through a reduction in N content. This reduction in N can decrease decomposition rates of SOM, as reflected in the litter C:N ratio, and indicates burning may cause the soil environment to become N limited. Also, burning had an effect on the lignin:N ratio, which can control rates of N mineralization and decomposition. The mineral soil appeared to buffer the effects of fire, as there was no effect of fire at 2.5 FPD, but SOM and SOC were drastically reduced at 5 FPD. The soil microbial community had a similar response to fire with a reduction in gram negative bacteria under 5 FPD and no difference under 2.5 FPD. Our hypothesis that the burned units would be more similar to each other than the control was not supported, as the 2.5 FPD treatment was more similar to the 0 FPD treatment. The largest effect of burning was alteration to the N content, which will alter decomposition rates depending on the amount of C and

lignin in the plant material. Changes in SOM, SOC, and the soil microbial community indicated the soil environment had the capacity to resist changes due to fire and that repetitive burning at high frequency can be detrimental to soil carbon stocks and certain bacteria.

From our results it was clear that prescribed burning at 2.5 and 5 FPD reduced the TN of the litter, and this has been found in previous studies (Bell and Binkley, 1989; Hernandez and Hobbie, 2008). Fire induces N volatilization, and N-constituents may be in higher demand if prescribed burning causes an increase in ecosystem productivity. However, differences in litter N were not reflected in soil N pools. A previous study in an oak savanna found that oak litter in burned plots had a lower percentage of N, and decomposed at a slower rate independent of local soil N availability (Hernandez and Hobbie, 2008). This suggests that oaks produced litter with lower N content, and decomposition was controlled by N immobilization from the litter rather than N demands from the soil. Litter chemistry differences in our study were also reflected in the C:N and lignin:N ratio. The higher C:N ratio means litter in burned units will decompose at a slower rate than litter in unburned units. But, the lignin:N ratio under 5 FPD was not significantly different from 0 FPD. These two ratios are commonly used as a proxy for decomposition rates. The lower lignin:N ratio in 5 FPD may due to a greater amount of forbs and legumes found in the understory with increased fire in the OWMA (Burton, 2009). It has been reported that burning in oak savannas can cause an ecosystem to become N limited due to volatilization of N and low plant tissue quality leading to slower decomposition rates (Reich *et al.*,

2001). Though mineralization rates were not measured in our study, it may be true that our burned plots are more N limited than the unburned; causing plants to produce lower quality litter, and decomposition is slowed.

Soil organic matter and SOC response to fire depends on fire intensity, soil moisture, soil type, and vegetation (Gonzalez-Perez *et al.*, 2004). These factors make it difficult to generalize the response of SOM and SOC to fire. Previous studies have shown SOC to respond variably to prescribed burning at different frequencies. Annual burning in an oak forest decreased SOM and increased bulk density when compared to 0 and 2 FPD treatments (Phillips *et al.*, 2000). In *Quercus coccifera* shrublands no significant differences in SOC were observed with burn frequencies between 0 and 1.9 FPD (Ferran *et al.*, 2005). A study in an oak-pine forest reported no differences in SOC across several different burn frequencies over a 12-year period (Neill *et al.*, 2007). No differences were observed in the amount of SOC among fire frequencies that were roughly 0.83 to 3.3 FPD. My results followed the same trend where a medium burn frequency showed no reduction in SOC or SOM, and reduction in SOM or SOC was associated with an increase in bulk density. Phillips *et al.*, (2000) observed annual burning to decrease SOM by 31-41% depending on the year measured. Our results indicated a much more drastic decrease in SOM with higher frequency burning. Though these studies indicated the same overall trend of increased fire on soil, local variables appeared to have strong control over the loss of SOM and SOC. Our results concurred with earlier findings and

indicated burning at 5 FPD can dramatically alter soil physical and chemical properties.

Fire is known to alter soil microbial communities directly through heat and indirectly by altering substrate chemistry or availability. Immediately after a fire, soil microbial biomass is greatly reduced due to heat-induced death or toxic compounds produced through combustion (Certini, 2005). A previous study suggested low intensity prescribed burning could cause moderate changes to the soil microbial community in savannas and forests likely due to changes in nutrient and substrate availability (Ponder *et al.*, 2009). Long term shifts in the community due to repetitive burning have been less studied. Our results indicated that low intensity prescribed burning affects the soil microbial community, though the cause of these community differences was not clear. Our sampling was conducted three growing seasons following the last burn, allowing the microbial community to reestablish after the initial heat-induced death. Therefore, differences in soil microbial communities were presumably indirectly related to burning through changes to nutrient or substrate availability. There were no strong correlations between soil nutrients or soil physical properties with the abundance of gram negative bacterial PLFAs (data not shown). Nutrients or specific organic compounds not measured in this study could have a greater effect on the gram negative bacteria. Still, our results clearly show that prescribed burning at certain frequencies drives soil microbial communities to differentiate within the same landscape. The technique we used to assay the soil microbial community did not give us fine enough resolution to identify specific

bacteria that play known roles in different nutrient cycle processes. However, the shift in gram negative bacterial PLFA abundance means changes are occurring at broad taxonomic levels that may alter the ecosystem in ways we did not measure.

Prescribed burning is a necessary technique for the proper management of forest ecosystems. Our results show that managing for different ecosystem services with prescribed burning can occur while managing for carbon sequestration. It is apparent that burning can reach levels that not only cause large changes in soil physiochemical properties, but have a significant effect on the soil microbial community as well. Microbial community shifts can affect local fertility and ecosystem functioning as a whole. Through prescribed burning oak forests can be conserved and other ecosystem services provided, but it must be instituted carefully as to not destabilize SOC pools and the soil ecosystem.

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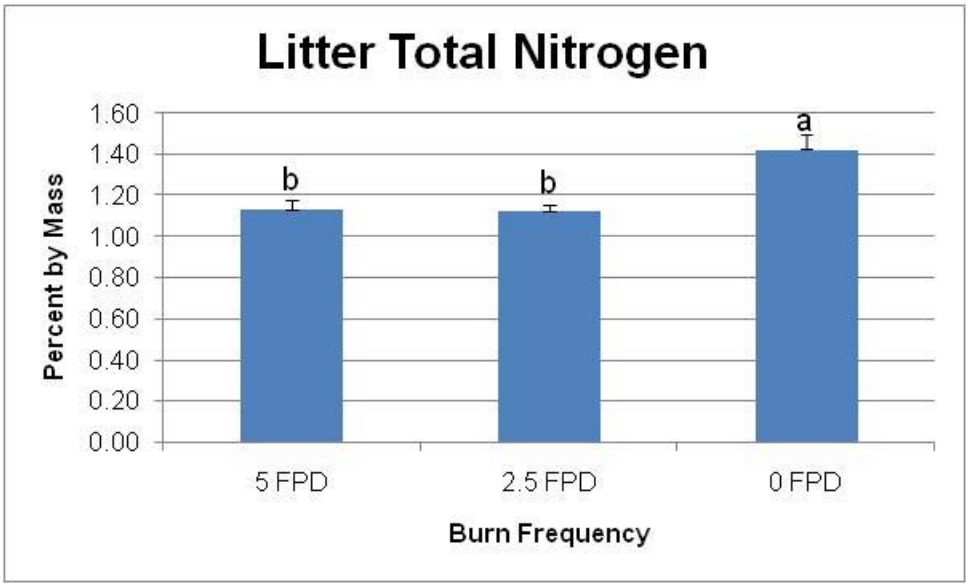
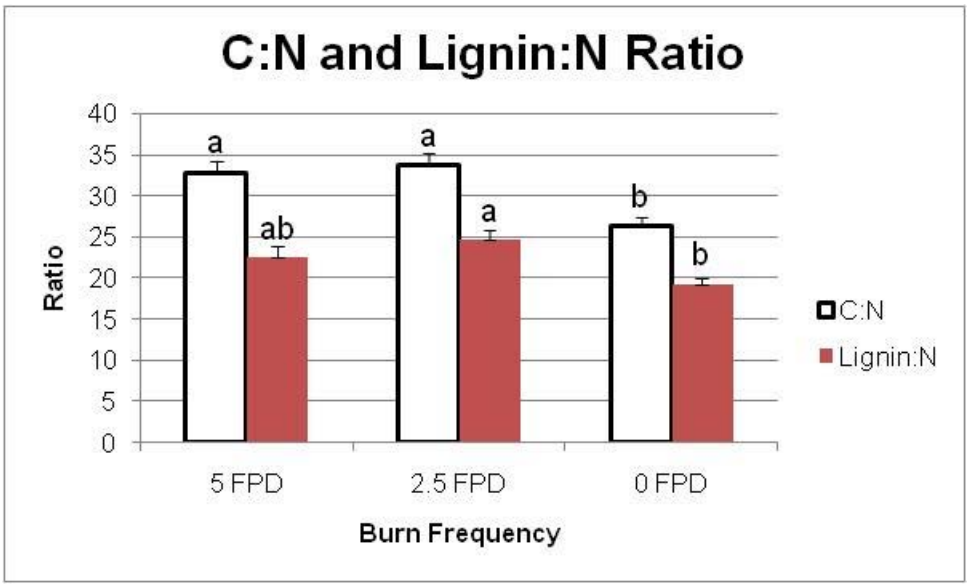
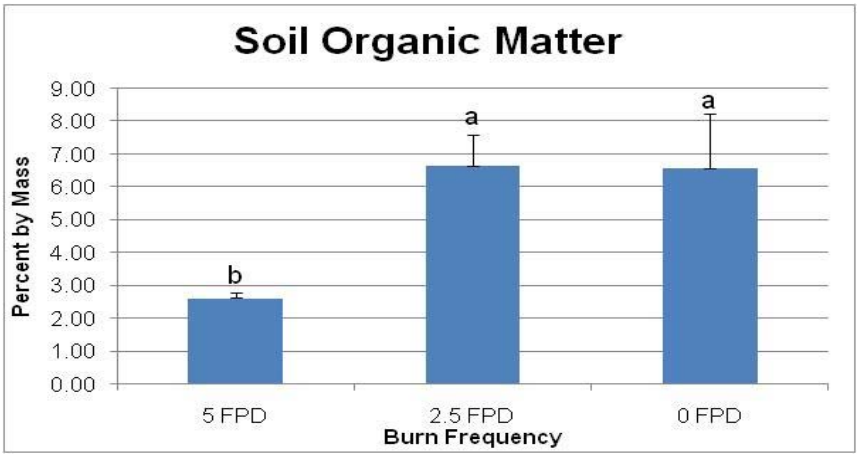
Figure 1. Litter nitrogen, C:N ratio, and lignin:N ratio. Bars represent the standard error. Mean separation is displayed by letters above standard error bars.

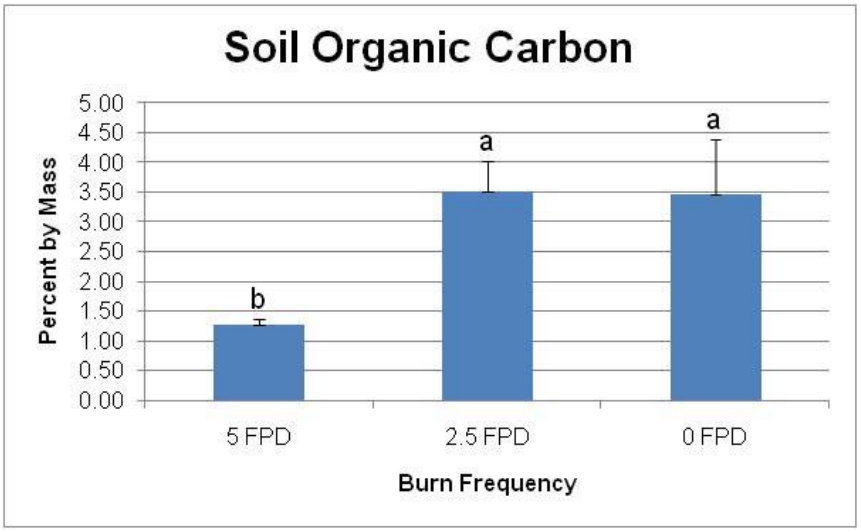
Figure 2. SOM, SOC, and soil bulk density. Bars represent the standard error. Mean separation is displayed by letters above standard error bars.

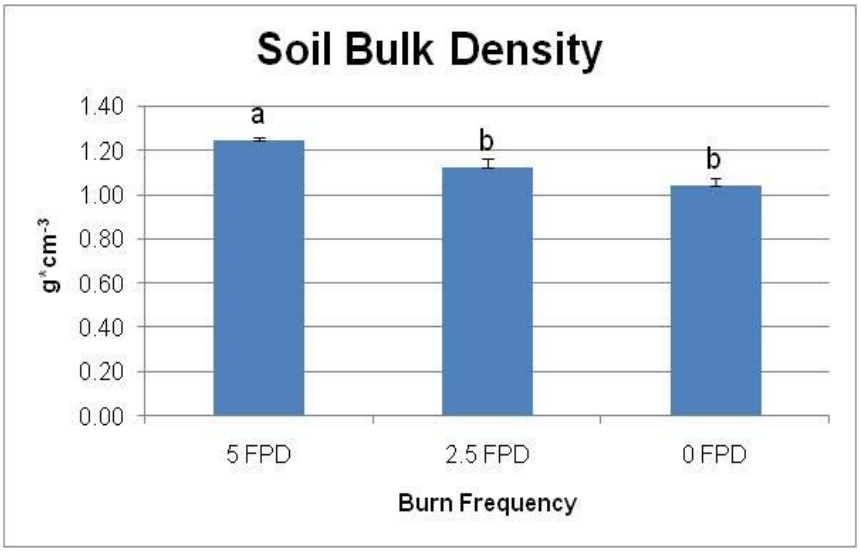
Figure 3. Gram negative bacteria PLFA biomarker abundance. Bars represent the standard error. Mean separation is displayed by letters above standard error bars.

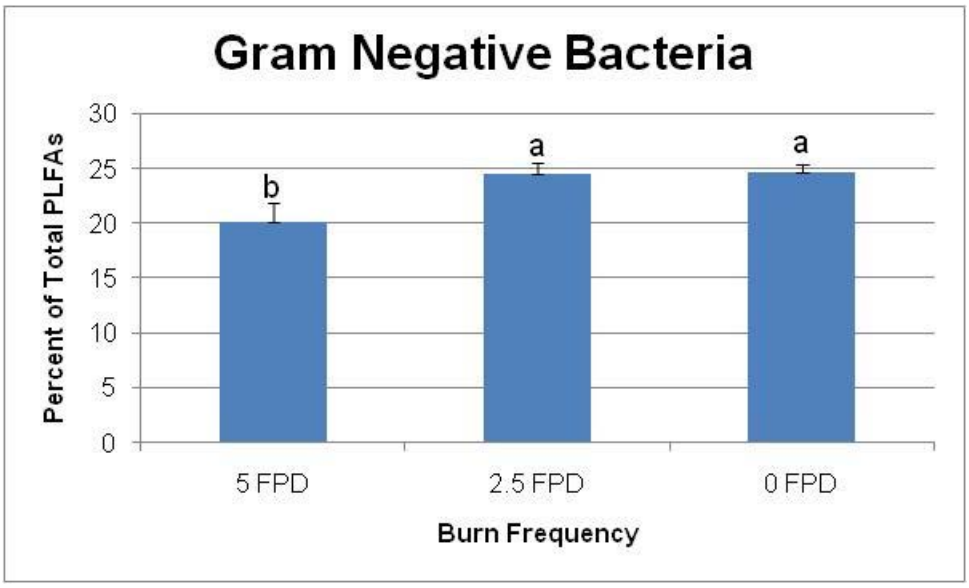

Litter Total Nitrogen
Percent by Mass
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
b
b
a
5 FPD
2.5 FPD
0 FPD
Burn Frequency


C:N and Lignin:N Ratio
Ratio
40
35
30
25
20
15
10
5
0
a
ab
a
a
b
b
5 FPD
2.5 FPD
0 FPD
Burn Frequency
C:N
Lignin:N


Soil Organic Matter
Percent by Mass
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
a
a
b
5 FPD
2.5 FPD
0 FPD
Burn Frequency


Soil Organic Carbon
Percent by Mass
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
a
a
b
5 FPD
2.5 FPD
0 FPD
Burn Frequency


Soil Bulk Density
g*cm-3
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
a
b
b
5 FPD
2.5 FPD
0 FPD
Burn Frequency


Gram Negative Bacteria
Percent of Total PLFAs
30
25
20
15
10
5
0
b
a
a
5 FPD
2.5 FPD
0 FPD
Burn Frequency

VITA

Ryan Williams

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF EASTERN REDCEDAR AND PRESCRIBED BURNING ON THE SOIL ECOSYSTEM OF THE CROSS TIMBERS FOREST

Major Field: Natural Resource Ecology and Management

Biographical:

Personal: Born in Plano, Texas on August 3rd, 1985, raised in Plano, Texas until the age of 18. Parents are Mr. James Barry Williams and Mrs. Susan Williams.

Education: Completed the requirements for the Bachelor of Science in Biology at Texas A&M University, College Station, Texas in December, 2007; completed the requirements for the Master of Science in Natural Resource Ecology and Management at Oklahoma State University, Stillwater, Oklahoma in July, 2010.

Experience: Employed as a research assistant in the Biology Department at Texas A&M University from December 2007 to August 2008; employed as a graduate research assistant in the Department of Natural Resource Ecology and Management at Oklahoma State University from August 2008 through July 2010.

Name: Ryan Williams Date of Degree: July, 2010

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: THE EFFECT OF EASTERN REDCEDAR AND PRESCRIBED BURNING ON THE SOIL ECOSYSTEM OF THE CROSS TIMBERS FOREST

Pages in Study: 73 Candidate for the Degree of Master of Science

Major Field: Natural Resource Ecology and Management

Scope and Method of Study: The purpose of this study was to explore the effect of two ecosystem factors, eastern redcedar (*Juniperus virginiana*) encroachment and prescribed burning, on the soil ecosystem of the Cross Timbers. I studied eight post oak *(Quercus stellata*) forests across central and western Oklahoma severely encroached by eastern redcedar. We sampled in areas dominated by eastern redcedar, post oak, and an even mix of both species. To determine the effect of prescribed burning on Cross Timbers soil, I studied stands burned with 0, 2.5 and 5 fires per decade (FPD). For both studies I analyzed litter nutrients, soil physiochemical properties, and soil microbial communities using phospholipid fatty acid analysis (PLFA).

Findings and Conclusions: Forest stands dominated by eastern redcedar had a lower litter carbon concentration than post oak-dominated stands, while mixed stands had a lower litter nitrogen concentration than post oak-dominated stands. I did not observe any effect of tree species on soil physiochemical properties. Partial principle component analysis and partial redundancy analysis indicated differentiation of soil microbial community composition in stands of each species. Eastern redcedar stands had notably higher amounts of arbuscular mycorrhizae fungal (AMF) PLFA biomarkers than post oak stands. AMF has a symbiotic relationship with eastern redcedar and inhibits the growth of ectomycorrhizae that have a symbiosis with post oak. These differences illustrated the potential for eastern redcedar to alter nutrient cycling through litter quality and develop a competitive advantage over post oak due to its increasingly abundant fungal symbiont. In the prescribed burn study, I found that litter had a lower nitrogen concentration, higher C:N ratio, and higher lignin:N ratio with any burning. The 5 FPD treatment had a third of the soil organic carbon concentration and a lower abundance of gram negative bacteria than the 2.5 and 0 FPD units. Results suggested that any burning reduced litter nitrogen which could lead to slowed decomposition and a nitrogen-limited soil ecosystem. High frequency prescribed burning altered soil microbial communities through shifts in substrate and nutrient availability that could potentially alter biogeochemical cycling within the forest.

ADVISER'S APPROVAL: Stephen W. Hallgren